

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
17 April 2003 (17.04.2003)

PCT

(10) International Publication Number
WO 03/030958 A1

(51) International Patent Classification⁷: **A61L 27/38**,
C08J 7/18

Pharmaceutical Sciences, The University of Nottingham,
Nottingham NG7 2RD (GB).

(21) International Application Number: PCT/GB02/04486

(74) Agent: **HARRISON GODDARD FOOTE**; 31 St.
Saviourgate, York YO1 8NQ (GB).

(22) International Filing Date: 3 October 2002 (03.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0124062.1 6 October 2001 (06.10.2001) GB
0219544.4 22 August 2002 (22.08.2002) GB

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK,
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GV, ML, MR, NE, SN, TD, TG).

(71) Applicants (*for all designated States except US*): **CELL-
TRAN LIMITED** [GB/GB]; Firth Court, Western Bank,
Sheffield S10 2TN (GB). **THE UNIVERSITY OF NOT-
TINGHAM** [GB/GB]; University Park, Nottingham NG7
2RD (GB).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **HADDOW, David**
[GB/GB]; Celltran Limited, Firth Court, Western Bank,
Sheffield S10 2TN (GB). **MACNEIL, Sheila** [GB/GB];
Celltran Limited, Firth Court, Western Bank, Sheffield S10
2TN (GB). **SHAKESHEFF, Kevin** [GB/GB]; School of

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: CORNEAL GRAFT

(57) Abstract: This invention relates to a contact lens which comprises a surface adapted by the provision of a plasma polymerised surface to which cells can attach, proliferate and detach to repair corneal lesions and the use of said contact lens in the treatment of eye conditions.

WO 03/030958 A1

CORNEAL GRAFT

The invention relates to a contact lens which is adapted by the provision of an acid
5 containing surface which is obtainable by plasma polymerisation and the use of said
lens in the repair of eye tissue, typically the cornea.

The ability to see is dependent on the actions of several structures in and around the
eye. When one focuses on an object, light rays are reflected from the object to the
10 cornea. The light rays are bent, refracted and focused by the cornea, lens, and
vitreous within the eye ball. The lens functions to ensure that light is focused on the
retina. The retina then converts light into electrical impulses which are transmitted
through the optic nerve to the brain where the image is perceived. The cornea is a
highly organised structure being composed of cells and proteins.

15

The cornea does not contain its own direct blood supply to nourish or protect it from
infection. This is because the presence of blood vessels would impair the passage of
light to the retina. The cornea is composed of five layers. The first of these is the
epithelium. The epithelium is the outer most layer and functions primarily to block
20 the passage for foreign bodies into the eye. It also provides a surface that absorbs
oxygen and cell nutrients from tears. Below the epithelium is the so called
Bowman's layer. It is composed of collagen and provides an attachment site for the
epithelium. Beneath the Bowman's layer is the stroma which comprises cells,
collagen and also a high percentage of water. Below the stroma is a membrane
25 referred to as Descemet's membrane. This membrane is also composed of collagen
but of a different type from that found in the stroma. The collagen is secreted by the
endothelial cells which lie below it. The endothelium is the inner most layer of the
cornea and functions to keep the cornea clear. It achieves this by removing excess
fluid from the stroma to prevent distortion and opaqueness in the cornea. The
30 endothelial cells provide an essential function and if the cells are lost either by

disease or trauma they are not replaced. If the endothelial layer is destroyed then the only corrective therapy is corneal transplantation.

5 There are a large number of diseases and conditions which affect the function of the cornea many of which do not require surgery. However, there are serious conditions which do require remedial action which includes surgery and the transplantation of corneal tissue.

10 For example, Fuchs' dystrophy is a progressive disease which is more common in women than in men. Fuchs' dystrophy occurs when endothelial cells are lost resulting in inefficient removal of liquid from the stroma. This causes the cornea to swell and distort vision. Ultimately the epithelial layer begins to swell resulting in abnormal curvature of the eyeball resulting in further distortion of vision. The epithelial swelling also produces scarring on the corneal surface. The initial treatment
15 of the disease is the use of drops, ointments or soft contact lenses to alleviate the symptoms. As the disease progresses the only option is a corneal transplant to restore sight.

A further disease affecting the cornea is Iridocorneal Endothelial Syndrome. This
20 disease is common in women and results in changes in colour of the iris, swelling of the cornea and the development of glaucoma. The disease is usually only present in one eye. The syndrome is defined by a group of three linked conditions referred to as iris nevus syndrome; Chandler's syndrome or essential iris atrophy. However a common feature of this group of diseases is the migration of endothelial cells off the
25 cornea and onto the iris. The loss of endothelial cells from the cornea results in corneal swelling and distortion of the iris with distortion of vision. The cause of the disease is unknown, the treatment includes the use of medication and ultimately corneal transplantation to control corneal swelling.

30 Keratoconus is a disorder resulting in the progressive thinning of the cornea. The disease begins with a thinning of the middle of the cornea which gradually bulges

outward resulting in an abnormal curvature. The disease is thought to have at least four origins. Keratoconus can be inherited since sufferers tend to have a family history of the disease. The disease can also occur as a result of eye injury or as a consequence of other diseases either of the eye (for example, retinitis pigmentosa, vernal kerato conjunctivitis) or other diseases which are not in themselves related to the eye but result in keratoconus, (for example Lebers congenital amaurosis, Ehleres-Danlos syndrome and osteogenesis imperfecta). In a majority of cases the cornea will stabilise without causing severe vision problems. However in a percentage of sufferers the cornea will eventually become too scarred and if this occurs the only therapy is corneal transplantation.

A yet further example of a disease resulting in corneal damage is lattice dystrophy which results in the accumulation of amyloid deposits or abnormal protein fibres in the stroma. The result of this is an increase in opaqueness resulting in reduced vision. In severe cases this can result in erosion of the outer epithelial layer resulting in a condition known as epithelial erosion. This results in abnormal cornea curvature resulting in loss of vision. This also results in exposure of nerves causing severe pain. A doctor can prescribe eye drops and ointments to reduce the erosion of the cornea. However, if the scarring becomes severe a corneal transplant may be needed. The replacement of the cornea is often only a temporary measure since the donor cornea has a high risk of contracting the disease within as little as three years. This means that the patient has to undergo a further corneal transplantation with the concomitant discomfort and stress.

The cornea can also be damaged through infection by a number of pathogenic agents. For example ocular herpes is a viral infection caused by herpes simplex virus and is a common cause of corneal blindness. Ocular herpes causes corneal inflammation and can be controlled by using antiviral drugs to inhibit viral replication and thereby reduce the destruction of the epithelial cells which the virus infects. However if the infection spreads into the other layers of the cornea a condition called stromal keratitis results which causes the immune system to attack and destroy stromal cells.

This condition is much more difficult to treat and ultimately results in scarring of the cornea with loss of vision and ultimately blindness. If the disease progresses to this stage then corneal transplantation is the only therapy.

- 5 Trachoma is a chronic inflammatory disease caused by a bacterial infection the causative agent of which is *Chlamydia trachomatis*. The disease is a progressive disease which begins in childhood and results in corneal scarring. The scarring results from eyelashes turning in and rubbing against the cornea resulting in corneal damage. The scarring results in severe vision loss and blindness, usually when
10 people are 40 to 50 years old. The disease is more common in the developing world affecting more than a 150 million people and blinding around 6 million per year in Asia and North Africa. The current treatment is the use of antibiotics to treat the infection. However people become re-infected and over the years scar tissue builds up in the cornea resulting in blindness. The only corrective measure then is corneal
15 transplantation.

Also, it is well known in the art that eyes are particularly susceptible to chemical burns caused by acid based chemicals (eg muriatic acid, sulphuric acid found in
batteries) and alkali based chemicals (e.g. lime, oven cleaners, ammonia). In severe
20 cases the cornea becomes scarred to the extent that the only corrective measure is corneal transplantation.

In our co-pending application, WO0078928, which is incorporated by reference, we disclose therapeutic vehicles which are adapted by the provision of surfaces which
25 contain acid functionality. These vehicles have utility in tissue engineering, particularly in the repair of cutaneous wounds. Advantageously, cells which attach to these surfaces proliferate and detach from the vehicle to invade the surrounding tissue to repair the wound. The surfaces are prepared by a method referred to as plasma polymerisation.

30

Plasma polymerisation is a technique which allows an ultra-thin (eg ca.200nm) cross linked polymeric film to be deposited on substrates of complex geometry and with controllable chemical functionality. As a consequence, the surface chemistry of materials can be modified, without affecting the bulk properties of the substrate so treated. Plasmas or ionised gases are commonly excited by means of an electric field. They are highly reactive chemical environments comprising ions, electrons, neutrals (radicals, metastables, ground and excited state species) and electromagnetic radiation. At reduced pressure, a regime may be achieved where the temperature of the electrons differs substantially from that of the ions and neutrals. Such plasmas are referred to as "cold" or "non-equilibrium" plasmas. In such an environment many volatile organic compounds (eg volatile alcohol containing compounds, volatile acid containing compounds, volatile amine containing compounds, or volatile hydrocarbons, neat or with other gases, eg Ar, have been shown to polymerise (H.K. Yasuda, Plasma Polymerisation, Academic Press, London 1985) coating both surfaces in contact with the plasma and those downstream of the discharge. The organic compound is often referred to as the "monomer". The deposit is often referred to as "plasma polymer". The advantages of such a mode of polymerisation potentially include: ultra-thin pin-hole free film deposition; plasma polymers can be deposited onto a wide range of substrates; the process is solvent free and the plasma polymer is free of contamination. Under conditions of low power, plasma polymer films can be prepared which retain a substantial degree of the chemistry of the original monomer. For example, plasma polymerised films of acrylic acid contain the carboxyl group (O'Toole L., Beck A.J., Short R. D., Macromolecules, 1996, 29, 5172-5177). The low power regime may be achieved either by lowering the continuous wave power, or by pulsing the power on and off (Fraser S., Barton D, Bradley J.W., Short R.D, J. Phys. Chem. B., 2002, 22 (106) 5596-5608).

Co-polymerisation of one or more compounds having functional groups with a hydrocarbon allows a degree of control over surface functional group concentrations in the resultant plasma copolymer (PCP) (Beck, A.J, Jones F.R.,

Short R.D., Polymer 1996, 37(24) 5537-5539). Suitably, the monomers are ethylenically unsaturated. Thus the functional group compound maybe unsaturated carboxylic acid, alcohol or amine, for example, whilst the hydrocarbon is suitably an alkene. By plasma polymerisation, it is also possible
5 to deposit ethylene oxide-type molecules (eg. tetraethyleneglycol monoallyl ether) to form 'non-fouling' surfaces. It is also possible to deposit perfluoro-compounds (i.e. perfluorohexane, hexafluoropropylene oxide) to form hydrophobic/superhydrophobic surfaces. This technique is advantageous because the surfaces have unique chemical and physical characteristics. Moreover, the
10 surface wettability, adhesion and frictional/wear characteristics of the substrate can be modified in a controllable and predictable manner.

We have cultured corneal epithelial cells on contact lenses which have been coated with plasma polymer/co-polymer. The use of a low plasma power/monomer (W/F)
15 flow rate ratio produces a plasma polymer/co-polymer in which the acid functionality of the acid-containing monomer (in this example, acrylic acid) is largely preserved intact (retained) from the plasma-gas to the plasma polymer/co-polymer deposit. These deposits do contain other functional groups (e.g. hydroxyls arising from post plasma oxidation) but are described in WO0078928 as "high acid
20 retention", reflecting the high degree of acid retention from the plasma gas into the plasma polymer film.

References herein to include surfaces which have amounts of 2-20% surface acid or 5-20% surface acid and in excess of 20% surface acid. The percentages refer to the
25 percent of carbon atoms in this type of environment. For example, 20 % acid means that 20 of every one hundred carbons in the plasma polymer is in an acid-type environment.

The present application relates to the provision of a structure, typically referred to as
30 a contact lens, which has been treated by plasma polymerisation to provide a surface

to which cells can readily attach and detach when the structure is placed against the eye.

According to an aspect of the invention there is provided a contact lens comprising
5 at least one surface wherein said surface is obtainable by plasma polymerisation.

In a preferred embodiment of the invention said surface is at least 2% acid.

In a further preferred embodiment of the invention said surface is at least 5% acid.
10

The phrase "contact lens" is not meant to be limiting with respect to the form or composition of the lens, rather it is meant to be descriptive of a structure which is adapted to intimately contact the eye to effect delivery of material attached to the surface (eg cells and/or therapeutic agents) of said lens, to the cornea or associated
15 eye structures. Contact lenses are typically disc shaped with a curvature adapted to fit about the outer surface of the eye ball. Also, contact lenses may be "soft" (i.e. are hydrophobic (i.e. not water containing) containing carbon and oxygen only (in approximately C:O = 75%:25%)) or "hard" depending on the nature of the material from which the lens is manufactured.

20

In an alternative preferred embodiment of the invention said surface has been treated by plasma polymerisation with a volatile acid.

In a preferred embodiment of the invention said surface has been treated by plasma
25 polymerisation with a volatile alcohol.

In a further alternative preferred embodiment of the invention said surface has been treated by plasma polymerisation with a volatile amine.

30 In a still further preferred embodiment of the invention said surface has been treated by plasma polymerisation with a mixture of volatile acid and volatile hydrocarbon.

In a preferred method of the invention said cell culture surface comprises a polymer comprising an acid content of at least 2%. Preferably said acid content is 2-20% or 5-20%. Alternatively, said acid content is greater than 20%. The percentages refer to
5 the percent of carbon atoms in this type of environment. The acid content of a contact lens surface is determined by methods herein disclosed and are known in the art. For example, percent acid maybe measured by x-ray photoelectron spectroscopy (XPS).

10 Polymerisable monomers that may be used in the practice of the invention preferably comprise unsaturated organic compounds such as, olefinic carboxylic acids and carboxylates, olefinic amines, olefinic alcohols, olefinic nitrile compounds, oxygenated olefins, halogenated olefins and olefinic hydrocarbons. Such olefins include vinylic and allylic forms. The monomer need not be olefinic, however, to be polymerisable.
15 Cyclic compounds such as cyclohexane, cyclopentane and cyclopropane are commonly polymerisable in gas plasmas by glow discharge methods. Derivatives of these cyclic compounds, such as 1, 2- diaminocyclohexane for instance, are also commonly polymerisable in gas plasmas. Particularly preferred are polymerisable monomers containing hydroxyl, amino or carboxylic acid groups. Of these,
20 particularly advantageous results have been obtained through use of acrylic acid or allyl amine. Mixtures of polymerisable monomers may be used. Additionally, polymerisable monomers may be blended with other gases not generally considered as polymerisable in themselves, examples being argon, nitrogen and hydrogen. The polymerisable monomers are preferably introduced into the vacuum chamber in the
25 form of a vapour. Polymerisable monomers having vapour pressures less than 1.3×10^{-2} mbar (1.3 Pa) are not generally suitable for use in the practice of this invention.

Polymerisable monomers having vapour pressures of at least 6.6×10^2 mbar (6.6 Pa) at ambient room temperature are preferred. Where monomer grafting to plasma
30 pressures of at least 1.3 mbar (130Pa) at ambient conditions are particularly preferred polymerisate deposits is employed, polymerisable monomers having vapour.



To maintain desired pressure levels, especially since monomer is being consumed in the plasma polymerisations operation, continuous inflow of monomer vapour to the plasma zone is normally practiced. When non polymerisable gases are blended with the monomer vapour, continuous removal of excess gases is accomplished by
5 simultaneously pumping through the vacuum port to a vacuum source, indeed this is the case when employing polymerisable monomers. Since some non-polymerisable gases are often evolved from glow discharge gas plasmas, it is advantageous to control gas plasma pressure at least in part through simultaneous vacuum pumping during plasma polymerisate deposition on a substrate in the process of this invention.

10

Monomers suited for this invention include, fully saturated and unsaturated carboxylic acid compounds up to 20 carbon atoms. More typically 2-8 carbons. Ethylenically unsaturated compounds (especially α,β unsaturated carboxylic acids) including acrylic acid, methacrylic acid. Saturated including ethanoic acid and
15 propanoic acid. Alternatively, compounds that can be plasma polymerised that readily hydrolyse to give carboxylic acid functionalities, e.g. organic anhydrides (e.g. maleic anhydride) acyl chlorides may be used.

In a further preferred method of the invention said polymer comprises an acrylic acid
20 monomer with at least 2% or 5% acid content. Preferably said acid content is between 2% and 20% or between 5% and 20%. Alternatively, the acid content can be $> 20\%$.

In a further preferred method of the invention said polymer comprises an acid co-
25 polymer. The copolymer is prepared by the plasma polymerisation of an organic carboxylic acid (or anhydride) with a saturated (alkane) or unsaturated (alkene, diene or alkyne) hydrocarbon. The hydrocarbon would be of up to 20 carbons (but more usually of 4- 8). Examples of alkanes are butane, pentane and hexane. Examples of alkenes are butene and pentene. An example of a diene is 1-7 octadiene-. The co-
30 monomer may also be aromatic-containing e.g. styrene.

Co-plasma polymerisation may be carried out using any ratio of acid : hydrocarbon, but will be typically using an acid: hydrocarbon ratio between the limits of 100(acid):0(hydrocarbon) to 20 (acid):80 (hydrocarbon) and any ratio between these limits.

5

Plasma polymerised amines are also within the scope of the invention, for example, fully saturated primary, secondary or tertiary amines (e.g. butyl amine, propyl amine, heptylamine) or unsaturated e.g, allyl amine, which are at least 20 carbons but more typically 4-8 carbons. Amines could be co-polymerised with hydrocarbons as

10 above.

The glow discharge through the gas or blend of gases in the vacuum chamber may be initiated by means of an audiofrequency, a microwave frequency or a radiofrequency field transmitted to or through a zone in the vacuum chamber. Particularly preferred
15 is the use of a radiofrequency (RF) discharge, transmitted through a spatial zone in the vacuum chamber by an electrode connected to an RF signal generator. A rather broad range of RF signal frequencies starting as low as 50 kHz may be used in causing and maintaining a glow discharge through the monomer vapor. In commercial scale usage of RF plasma polymerisation, an assigned radiofrequency of
20 13.56 MHz may be more preferable to use to avoid potential radio interference problems as with examples given later. Typically, using the composite ratio of W/FM , as described by Yasuda (1985) the power loading should be $< 10^9$ J/kg to achieve functional group retention in plasma polymers. (W = power (J/min); F = flow rate (mole/min); M = average molecular mass (kg/mol) [Although flow rate is given
25 as sccm, this is in fact not strictly correct. Conversion from sccm to mol/min can be readily performed by dividing sccm by 22, 400).

The glow discharge need not be continuous, but may be intermittent in nature during plasma polymerisate deposition. Or, a continuous glow discharge may be employed,
30 but exposure of a substrate surface to the gas plasma may be intermittent during the overall polymerisate deposition process. Or, both a continuous glow discharge and a continuous exposure of a substrate surface to the resulting gas plasma for a desired

overall deposition time may be employed. The plasma polymerisate that deposits onto the substrate generally will not have the same elemental composition as the incoming polymerisable monomer (or monomers). During the plasma polymerisation, some fragmentation and loss of specific elements or elemental groups naturally occurs. Thus, in the plasma polymerisation of acrylic acid, carboxyl content of the plasma polymerisate is typically lower than would correspond to pure polyacrylic acid. Similarly, in the plasma polymerisation of allylamine, nitrogen content of the plasma polymerisate is typically lower than would correspond to pure polyallylamine. Exposure time to either of these unreacted monomers in the absence of a gas plasma, as through intermittent exposure to a glow discharge, allows for grafting of the monomer to the plasma polymerisate, thereby increasing somewhat the level of the functional group (carboxylic acid or amine) in the final deposit. Time intervals between plasma exposure and grafting exposure can be varied from a fraction of a second to several minutes.

15

In still a further preferred embodiment of the invention, said surface is provided by coating at least one surface (e.g. the surface in contact with the eye) with a plasma co-polymer of an acidic monomer. For example and not by way of limitation, acrylic acid and a hydrocarbon, for example, 1,7-octadiene. Ideally said acrylic acid is provided at 50-100% and 1,7-octadiene is provided at 0-50% in the gas feed.

20

According to a further aspect of the invention there is provided the use of the contact lens according to the invention as a cell culture surface.

25 In a preferred embodiment of the invention said surface is for use in the culture of mammalian cells, preferably human cells.

In a preferred embodiment of the invention said surface is for use in the culture of cells derived from the cornea. Preferably said cells are selected from the group consisting of: corneal endothelial cells; corneal stromal cells; corneal epithelial cells;

30

corneal stem cells or stem cells with corneal potentiality; embryonic stem cells; or adult stem cells.

In a preferred embodiment of the invention said cell is autologous.

5

In an alternative preferred embodiment of the invention said cell is allogenic.

It will be apparent to one skilled in the art that the cells used to seed the contact lens according to the invention may be either derived from self (autologous) or non-self
10 (allogenic).

According to a yet further aspect of the invention there is provided a method to culture mammalian cells on a contact lens comprising the steps of:

- i) providing a preparation comprising;
 - 15 a) corneal derived cells;
 - b) a contact lens according to the invention and attached thereto, fibroblast feeder cells;
 - c) cell culture medium sufficient to support the growth of said mammalian cells; and
- 20 ii) providing cell culture conditions which promote the proliferation of said mammalian cells on said contact lens.

In a preferred method of the invention said medium does not include serum.

25 In a preferred method of the invention said mammalian cells are human.

In a further preferred method of the invention said fibroblast feeder cells are human.

In a further preferred method of the invention said fibroblast feeder cells are human
30 dermal fibroblasts or human oral fibroblasts. Preferably said feeder cells are autologous.

The direct culturing of mammalian cells on a contact lens according to the invention under conditions herein disclosed has obvious benefits in corneal repair since the fabrication of said contact lens allows the culturing, implantation and transfer of cells
5 to a corneal lesion to be repaired. The absence of serum and the use of autologous cells also minimizes the transfer of xenobiotic agents (e.g. viral agents, prions) from serum and/or feeder cells used in the culture of mammalian cells.

According to a further aspect of the invention there is provided a method to treat an
10 animal, preferably a human, suffering from an eye condition which would benefit from the application of the contact lens according to the invention comprising :

- i) seeding a lens according to the invention with at least one mammalian cell;
and
- 15 ii) contacting the seeded lens with an eye surface.

In a preferred method of the invention said eye condition is selected from the group consisting of the following: Fuchs' dystrophy; keratoconus; lattice dystrophy; map-dot-finger print dystrophy; iridocorneal endothelial syndrome; iris nevus (Cogan-
20 Reese) syndrome; Chandler's syndrome; essential iris atrophy; Stevens-Johnson syndrome.

In a further preferred method of the invention said eye condition is the result of an infection caused by herpes simplex virus.
25

In a yet further preferred method of the invention said eye condition is the result of an infection caused by *Chlamydia trachomatis*.

In a yet further preferred method said eye condition is the result of chemical burns.
30

In a further preferred method according to the invention the lens is seeded with a mammalian cell selected from the group consisting of: corneal endothelial cells; corneal stromal cells; corneal epithelial cells; corneal stem cells or stem cells with corneal potentiality; embryonic stem cells; or adult stem cells.

5

According to a further aspect of the invention there is provided a method to coat a contact lens comprising the steps of:

- i) providing at least one organic monomer (e.g. acrylic acid);
- ii) creating a plasma of said organic monomer; and
- 10 iii) coating at least one lens surface with said plasma.

In a preferred method of the invention said monomer is an acid monomer source comprising 30-99% acid monomer. Preferably said acid monomer source consists of a 100% acid monomer source. Preferably said method consists of a 100% acrylic
15 acid source.

According to a further aspect of the invention there is provided a method to treat a contact lens comprising the steps of:

- i) providing a selected ratio of a monomer and a hydrocarbon in a gas feed;
- 20 ii) creating a plasma of said mixture;
- iii) bringing into contact a contact lens with said plasma mixture to provide a contact lens comprising a co-polymer.

In a preferred method of the invention said monomer is an acid monomer and said
25 co-polymer is an acid co-polymer.

In a preferred method of the invention said plasma is created by means of electrical power input (radio frequency 13.56MHz), coupled by means of a copper coil or bands. The reactor volume is in the range 2- 10 L and the reactor is pumped by
30 means of a double stage rotary pump to a base pressure approaching 10^{-4} mbar. In the case of replacing the rotary pump with a turbomolecular pump better base pressures



can be achieved. The monomer pressure is in the range 10^{-1} mbar to 10^{-3} mbar and the monomer flow rate is 1-20 cm³/min. The power would be typically 0.5 –50W continuous wave. Those skilled in the art may adjust these parameters to produce like plasmas by pulsing on the micro or milli second time scales.

5

In a preferred method of the invention said acid is acrylic acid and said hydrocarbon is a diene and especially a di-unsaturated alkene, for example 1,7-octadiene.

In a further preferred method of the invention said plasma comprises 50-100% unsaturated acid, for example, acrylic acid and 0-50% hexane or diene, (for example, 1,7-octadiene) in the gas feed.

In yet a further preferred embodiment of the invention said plasma comprises the following ratios of acid (eg acrylic acid) and hexane or diene(eg1,7-octadiene);

15	Acid		alkene
	(eg Acrylic acid)	%	(eg 1,7-octadiene %)
	50		50
	60		40
	70		30
20	80		20
	90		10
	100		0

in the gas feed.

25 An embodiment of the invention will now described, by example only and with reference to the following materials and methods.

Materials and Methods

30 Plasma Polymerisation of an Acid Monomer

Acrylic acid was obtained from Aldrich Chemical Co. (UK). The monomer was aliquated into 5ml batches and stored in a refrigerator until required for use. For each polymerisation one 5ml aliquot was used and then discarded. Prior to polymerisation the monomer was degassed using several freeze-pump/thaw cycles. Polymerisation was carried out in a cylindrical reactor vessel (of 8cm diameter and 50 cm length), evacuated by a two stage rotary pump. Stainless steel flanges were sealed to the glass vessel using viton 'o' rings. The contact lenses were placed on a two tier stainless steel tray in the centre of the glass vessel. The plasma was sustained by a radio-frequency (13.56 MHz) signal generator and amplifier inductively coupled to the reactor vessel by means of an external copper coil. The base pressure in the reactor prior to polymerisation was always $< 1 \times 10^{-3}$ mbar.

Acrylic acid was polymerised using continuous wave plasma powers in the range of 1-10W and a total flow rates in the range 1-20W sccm Plasma polymers were deposited onto the contact lenses (which were positioned with the concave side facing upwards), and clean silica glass cover slips or Al foil for XPS analysis. The pressure with the monomer flowing was typically 4.0×10^{-2} mbar. A further co-polymerisation using acrylic acid and 1,7-octadiene was carried out using the same range of power, flow rate and pressure conditions.

20

For all polymerisations and co-polymerisations a deposition time of typically 15 minutes was used. The monomers were allowed to flow for typically a further 20 minutes after the plasma was extinguished in order to minimise the up-take of atmospheric oxygen by the deposits on exposure to the laboratory atmosphere.

25

The contact lenses were removed from the glass reactor and cover slips analysed by XPS. In order that the reactor vessel and steel sample tray were in a clean condition for each polymerisation, an etch with an oxygen plasma was carried out after each deposition. The oxygen gas was allowed to flow through the reactor at a pressure of 1.0×10^{-1} mbar. The plasma power was 50W and the etch time was one hour. XPS of

30



a silica glass cover slip that had previously been polymerised with acrylic acid was examined by XPS to confirm that all the acid had been etched away.

Plasma Polymerisation of Amine and Alcohol Monomers

5

Following the procedure described above for acrylic acid, the same reactor and plasma conditions (namely powers, pressures and flow rates) plasma polymers containing amine or alcohol functionality were prepared from allyl amine and allyl alcohol monomers, respectively (Aldrich Chemical Co, UK). Plasma polymers were deposited onto the concave side of contact lenses and clean silica glass coverslips or Al foil for XPS.

Copolymerisations using allyl amine or allyl alcohol and 1,7-octadiene were carried out in the same manner.

15

X-ray Photoelectron Spectroscopy

XP spectra were obtained on a VG CLAM 2 photoelectron spectrometer employing Mg K_{α} x-rays. Survey scan spectra (0-1100 eV) and narrow scan spectra of C and O were acquired for each sample using analyser pass energies of 50 and 20 eV respectively. Spectra were acquired using Spectra 6.0 software (R. Unwin Software, Cheshire, UK). Subsequent processing was carried out using Scienta Esca Analysis for Windows (Scienta Instruments, Uppsala, Sweden). The spectrometer was calibrated using the Au 4f 7/2 peak position at 84.0 eV, and the separation between the C 1s and F 1s peak positions in a sample of PTFE measured at 397.2 eV, which compares well with the value of 397.19 eV reported by Beamson and Briggs (ref:G.Beamson and D.Briggs, eds.,*High Resolution XPS of Organic Polymers: The Scienta ESCA300 Handbook*, John Wiley and Sons, Chichester, UK, 1992.).

Cell Culture

Donor corneas were obtained from Bristol Eye Bank, UK. A cornea donor punch (Barron Donor Punch, Katena Eye Instruments, USA) was used to remove a central 8mm section of the donor cornea. From the remaining peripheral tissue 2mm pieces
5 of tissue were cut from the limbal region. Cells were extracted from this tissue enzymatically using Trypsin and primary cultures were established on a feeder layer of irradiated 3T3 mouse fibroblasts. Cells were cultured in low calcium media – approximately $1/10^{\text{th}}$ physiological (0.1-0.2mm) using media such as Green's low calcium media for 10-16 days. At this point cells were used experimentally or for
10 clinical use by seeding onto a plasma coated contact lens.

In a clinical situation corneal epithelial cells may be obtained using a small 2mm biopsy from an unaffected area of the eye to be treated or from the contralateral eye. (This technique could also be used for allogenic cells but would require
15 immunosuppression of the patient for periods of several months if not longer).

Plasma coated contact lenses were seeded on the concave surface with cultured corneal epithelial cells at a range of cell densities. Cells were cultured on these lenses for periods of time from 1-7 days to establish viability and proliferation of
20 cells on lenses. The MTT-ESTA assay was used to measure dehydrogenase activity as an indicator of metabolic viability. For proliferation cells were digested from the lens using a buffer containing urea and detergent (SDS) to analyse DNA. Results showed that cells survived well and increased in number on these plasma coated lenses for several days.

25

Additionally colony forming potential of the cultured cells was examined by harvesting cells from the lens using trypsin and then seeding cells at low density (e.g. 1000 cells per 10cm diameter dish) onto a feeder layer of irradiated 3T3 mouse fibroblasts. Cells were left for 7 days and then cells forming successful colonies
30 were stained using rhodamine and also H&E.

In alternative strategies for expanding corneal epithelial cells, these were initially expanded on growth arrested human dermal fibroblasts (which could be autologous or allogeneic in origin) or oral fibroblasts (as before, autologous or allogeneic).

5

Use of autologous fibroblasts (obtained by punch biopsy from the oral mucosa or the skin and then trypsinisation of the biopsy and collagenase extraction of the fibroblasts) avoids the need to use animal derived cells for initial corneal epithelial cell expansion. Additionally culture of corneal epithelial cells on growth arrested autologous dermal or oral fibroblasts allows one to expand corneal epithelial cells in the absence of serum. Fibroblasts can be growth arrested by irradiation (in established use since the early 1980s for expansion of cutaneous keratinocytes for clinical use) or can be growth arrested by culture of both fibroblasts and corneal epithelial cells in low calcium media – this permits proliferation of corneal epithelial cells but not of dermal fibroblasts or oral fibroblasts as calcium concentrations of less than 0.3mM effectively arrest fibroblast proliferation. Hence a media containing calcium at around 0.15mM will allow epithelial cell expansion on growth arrested fibroblasts. This obviates the need to use gamma irradiation to growth arrest fibroblasts. Under these conditions, corneal epithelial cells increased in number to the same degree as that seen when cells were grown in the absence of fibroblasts and the presence of serum. Essentially, growth arrested fibroblasts are substituting for 10% fetal calf serum in this situation.

To assess corneal epithelial cell transfer from plasma coated contact lenses, the corneal cells were grown to relatively high density on the plasma coated contact lens using one of the methodologies as described. An *in vitro* corneal wound model was used consisting of a de-epithelialised cornea. Corneas obtained from the Bristol Eye Bank (UK) were treated with 1 molar sodium chloride at 4° overnight and then a scalpel was used to lightly cut through the cornea into the stroma describing a hexagon to encompass a central area of approximately 1cm diameter. Fine forceps

were then used to gently peel the corneal epithelium from the underlying stroma. In developing this model the removal of the epithelium was assessed by staining the cornea with MTT-ESTA – successful removal of the cornea could be readily seen using this technique which allows ready visualisation of metabolically active cells.

5

Once the corneal epithelium was removed from the cornea, the bandage contact lens (containing epithelial cells on its inner surface) was then fixed in place using either tissue glue or sutures. The cornea with lens in place could be cultured both at an air-liquid interface and in a submerged culture for 5 days. At the end of this period the
10 glue or sutures were removed using scissors and the contact lens gently removed from the cornea. The transfer of corneal epithelial cells from the lens to the cornea was assessed by staining cornea and lens with MTT-ESTA which allowed rapid visualisation of the location of the cells.

15 Histology of the epithelium on the cornea was also undertaken to assess the nature of the transferred epithelium and the extent to which it had formed a secure attachment to the underlying stroma.

The transfer of cells was assessed using both a standard serum containing protocol
20 (in which cells were cultured in the presence of 10% fetal calf serum) and also in a serum free protocol in which corneal cells were initially expanded under serum free conditions and then plated onto the plasma coated contact lens in the presence of growth arrested fibroblasts (to maintain the mitogenic drive necessary to ensure corneal epithelium proliferation). As before the source of fibroblasts could be
25 autologous, dermal or oral mucosa fibroblasts.

The major benefit in avoiding the use of serum currently lies in avoiding problems associated with detecting BSE. For this reason we used human rather than mouse fibroblasts in this study. For clinical use it will be necessary to expand fibroblasts
30 from the initial patient biopsy using serum-free defined media containing recombinant mitogens. In considering how cells attach to a surface it was interesting

to note that both fibroblasts attached successfully to the 10W plasma polymer surface under serum free conditions. Attachment of cells could be occurring directly to the surface or through a protein layer attached to the surface. When serum is present the protein coating the surface will be serum derived; in its absence the cells themselves
5 may secrete the proteins. A typical example is fibroblasts, which secrete large amounts of fibronectin in culture. Serum in contrast contains both adhesive (fibronectin and vitronectin) and anti-adhesive proteins (e.g. very large amounts of albumin). Serum also contains a range of platelet-derived mitogens such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming
10 growth factor (TGF- β) which all stimulate cell proliferation. It is because of these mitogens that serum is extensively used in cell culture. In producing defined media recombinant mitogens can be used.

15

20

25

30

35

40



Claims

1. A contact lens comprising a surface wherein said surface is obtainable by
5 plasma polymerisation.
2. A contact lens according to Claim 1 wherein said surface has an acid
content of at least 2% acid.
- 10 3. A contact lens according to Claim 1 wherein said surface has an acid content
of at least 5% acid.
4. A contact lens according to any of Claims 1-3 wherein said surface has been
treated by plasma polymerisation with a volatile acid.
- 15 5. A contact lens according to Claim 1 wherein said surface has been treated by
plasma polymerisation with a volatile alcohol.
6. A contact lens according to Claim 1 wherein said surface has been treated by
20 plasma polymerisation with a volatile amine.
7. A contact lens according to Claim 1 wherein said surface has been treated by
plasma polymerisation with a volatile hydrocarbon.
- 25 8. A contact lens according to Claim 1 wherein said surface has been treated by
plasma polymerisation with at least one molecule selected from the group consisting
of : acrylic acid, propionic acid, methacrylic acid, maleic anhydride, allyl alcohol,
octa-1,7-diene; or allyl amine.
- 30 9. A contact lens according to any of Claims 1-8 wherein said surface is
obtainable by plasma polymerisation of a monomer preparation.

10. A contact lens according to Claim 9 wherein said monomer preparation consists essentially of an ethylenically unsaturated organic compound.
11. A contact lens according to Claim 9 or 10 wherein said monomer preparation
5 comprises essentially of a single ethylenically unsaturated organic compound.
12. A contact lens according to any of Claims 9-11 wherein said monomer is selected from the group consisting of: an alkene; a carboxylic acid, an alcohol; or an amine.
- 10 13. A contact lens according to any of Claims 9-12 wherein the monomer preparation comprises a mixture of two or more ethylenically unsaturated organic compounds.
- 15 14. A contact lens according to Claim 13 wherein said compounds are selected from the group consisting of: an alkene; a carboxylic acid; an alcohol; or amine.
15. A contact lens according to Claim 1 wherein said plasma polymer is a co-polymer.
- 20 16. A contact lens according to Claim 15 wherein said co-polymer comprises at least one organic monomer and at least one hydrocarbon.
17. A contact lens according to Claim 16 wherein said organic monomer is
25 selected from the group consisting of: carboxylic acid; an alcohol or an amine.
18. A contact lens according to Claim 16 or 17 wherein said hydrocarbon is an alkane.
- 30 19. A contact lens according to Claim 16 or 17 wherein said hydrocarbon is an alkene.



20. A contact lens according to Claim 19 wherein said alkene is a diene.
21. A contact lens according to Claim 20 wherein said diene is octa 1,7-diene.
- 5 22. A contact lens according to Claim 1 wherein said surface acid is between 2
20% surface acid.
23. A contact lens according to Claim 1 wherein said surface acid is greater than
10 20% acid.
24. A contact lens according to Claim 16 or 17 wherein said co-polymer is of
acrylic acid and a hydrocarbon.
- 15 25. A contact lens according to Claim 16 or 17 wherein said co-polymer is of
allylamine and a hydrocarbon.
26. A contact lens according to Claim 16 or 17 wherein said co-polymer is of
allyl alcohol and a hydrocarbon.
- 20 27. A contact lens according to any of Claims 24-26 wherein said hydrocarbon
is 1,7 octadiene.
28. The use of a contact lens according to any of Claims 1-27 as a cell culture
25 surface.
29. Use according to Claim 28 wherein said use is in the culture of mammalian
cells.
- 30 30. Use according to Claim 29 wherein said mammalian cells are human cells.

31. Use according to Claim 29 or 30 wherein said mammalian cells are derived from the cornea.
32. Use according to Claim 31 wherein said cells are selected from the group
5 consisting of: corneal endothelial cells; corneal stromal cells; corneal epithelial cells; corneal stem cells or stem cells with corneal potentiality.
33. Use according to Claim 29 or 30 wherein said cells are embryonic stem cells.
- 10 34. Use according to any of Claims 29-33 wherein said cells are autologous.
35. Use according to any of Claims 29-33 wherein said cells are allogenic.
36. A method to culture mammalian cells on a contact lens comprising the steps
15 of:
- i) providing a preparation comprising;
- a) corneal derived cells;
- b) a contact lens according to any of Claims 1-27 and attached thereto, fibroblast feeder cells;
- 20 c) cell culture medium sufficient to support the growth of said mammalian cells; and
- ii) providing cell culture conditions which promote the proliferation of said mammalian cells on said contact lens.
- 25 37. A method according to Claim 36 wherein said medium does not contain serum.
38. A method according to Claim 36 or 37 wherein said mammalian cells are human.
- 30

39. A method according to any of Claims 36-38 wherein said fibroblast feeder cells are human.

40. A method according to Claim 39 wherein said feeder cells are dermal
5 fibroblasts

41. A method according to Claim 39 wherein said feeder cells are oral fibroblasts.

42. A method according to any of Claims 36-41 wherein said feeder cells are
10 autologous.

43. A method to treat an animal, preferably a human, suffering from an eye condition which would benefit from the use of a contact lens according to any of Claims 28 -35 comprising :

- 15 i) seeding a lens according to any of Claims 1-27 with a mammalian cell; and
ii) contacting the seeded lens with an eye surface.

44. A method according to Claim 43 wherein said eye condition is selected from the group consisting of: Fuchs' dystrophy; keratoconus; lattice dystrophy; map-dot-
20 finger print dystrophy; iridocorneal endothelial syndrome; iris nevus (Cogan-Reese) syndrome; Chandler's syndrome; essential iris atrophy; Stevens-Johnson syndrome.

45. A method according to Claim 44 wherein said eye condition is the result of an infection caused by herpes simplex virus.
25

46. A method according to Claim 43 wherein said eye condition is the result of an infection caused by *Chlamydia trachomatis*.

47. A method according to Claim 43 wherein said eye condition is the result of
30 chemical burns.

48. A method to coat a contact lens comprising the steps of:

- i) providing at least one organic monomer;
- ii) creating a plasma of said organic monomer; and
- iii) coating at least one lens surface with said plasma.

5

49. A method according to Claim 48 wherein said organic monomer is an acid monomer.

50. A method to coat a contact lens comprising the steps of:

- 10 i) mixing a selected ratio of an organic monomer and a hydrocarbon in a gas feed;
- ii) creating a plasma of said mixture; and
- iii) coating a contact lens with said plasma to provide a surface polymer/copolymer.

15

51. A method according to Claim 50 wherein said organic monomer is selected from the group consisting of: carboxylic acid; an alcohol or an amine.

52. A method according to Claim 51 wherein said carboxylic acid is acrylic acid.

20

53. A method according to Claim 51 wherein said alcohol is allyl alcohol.

54. A method according to Claim 51 wherein said amine is allyl amine.

25 55. A method according to any of Claims 50-54 wherein said hydrocarbon is a diene.

56. A method according to Claim 55 wherein said diene is a di-unsaturated alkene.

30

57. A method according to Claim 52 wherein said alkene is 1,7-octadiene.

58. A method according to any of Claims 50-57 wherein said plasma comprises the following ratios of acid and diene:

	Acid	diene
5	%	%
	50	50
	60	40
	70	30
	80	20
10	90	10
	100	0

in the gas feed.

15

20

25

30

35

INTERNATIONAL SEARCH REPORT

Internati application No

PCT/GB 02/04486

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/38 C08J7/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61L C08J A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, COMPENDEX, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 78928 A (HADDOW DAVID ;MACNEIL SHEILA (GB); SHORT ROBERT (GB); UNIV SHEFFIE) 28 December 2000 (2000-12-28) cited in the application claims	1-27, 48-58
X	LATKANY R. ET AL.: "PLASMA SURFACE MODIFICATION OF ARTIFICIAL CORNEAS FOR OPTIMAL EPITHELIALIZATION" JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, vol. 36, no. 1, July 1997 (1997-07), pages 29-37, XP002224793 USA abstract	1-27, 48-58



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

16 December 2002

Date of mailing of the international search report

23/01/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ESPINOSA, M

INTERNATIONAL SEARCH REPORT

Internati Application No

PCT/GB 02/04486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE COMPENDEX 'Online! ENGINEERING INFORMATION, INC., NEW YORK, NY, US; YASUDA H ET AL: "ULTRATHIN COATING BY PLASMA POLYMERIZATION APPLIED TO CORNEAL CONTACT LENS" Database accession no. EIX76010003844 XP002225081 abstract & J BIOMED MATER RES NOV 1975, vol. 9, no. 6, November 1975 (1975-11), pages 629-643,</p> <p>---</p>	1-27, 48-58
P,X	<p>CHU P K ET AL: "Plasma-surface modification of biomaterials" MATERIALS SCIENCE AND ENGINEERING R: REPORTS, ELSEVIER SEQUOIA S.A., LAUSANNE, CH, vol. 36, no. 5-6, 29 March 2002 (2002-03-29), pages 143-206, XP004343705 ISSN: 0927-796X abstract</p> <p>---</p>	1-27, 48-58
A	<p>PELLEGRINI G ET AL: "Location and Clonal Analysis of Stem Cells and their Differentiated Progeny in the Human Ocular Surface" THE JOURNAL OF CELL BIOLOGY, ROCKEFELLER UNIVERSITY PRESS, US, vol. 145, no. 4, 17 May 1999 (1999-05-17), pages 769-782, XP002192318 ISSN: 0021-9525 abstract</p> <p>---</p>	1-58
A	<p>WO 98 31316 A (CASTRO MUNOZLEDO FEDERICO ;CELADON SCIENCE LLC (US); KURI HARCUCH) 23 July 1998 (1998-07-23) claims; example</p> <p>---</p>	1-58
A	<p>PELLEGRINI G ET AL: "Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium" LANCET, XX, XX, vol. 349, no. 9057, 5 April 1997 (1997-04-05), pages 990-993, XP004267066 ISSN: 0140-6736 the whole document</p> <p>-----</p>	1-58

S

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/04486

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 43-47 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internati Application No

PCT/GB 02/04486

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0078928	A	28-12-2000	AU	5546400 A	09-01-2001
			CN	1357039 T	03-07-2002
			EP	1185621 A2	13-03-2002
			WO	0078928 A2	28-12-2000
			NO	20016184 A	21-02-2002
<hr/>					
WO 9831316	A	23-07-1998	AU	6242298 A	07-08-1998
			WO	9831316 A1	23-07-1998